

HELLER EHRLICH HITE & CAULFIELD  
Sheet 1 of 6  
RENILLA REINFORMIS FLUORESCENT PROTEINS,  
NUCLEIC ACIDS ENCODING THE FLUORESCENT  
PROTEINS AND THE USE THEREOF IN  
DIAGNOSTICS, HIGH THROUGHPUT SCREENING  
AND NOVELTY ITEMS.

Applicant: Bryan et al. Our Docket No.: 24729-0128  
Serial No. 09/808,898 Filed: March 15, 2001

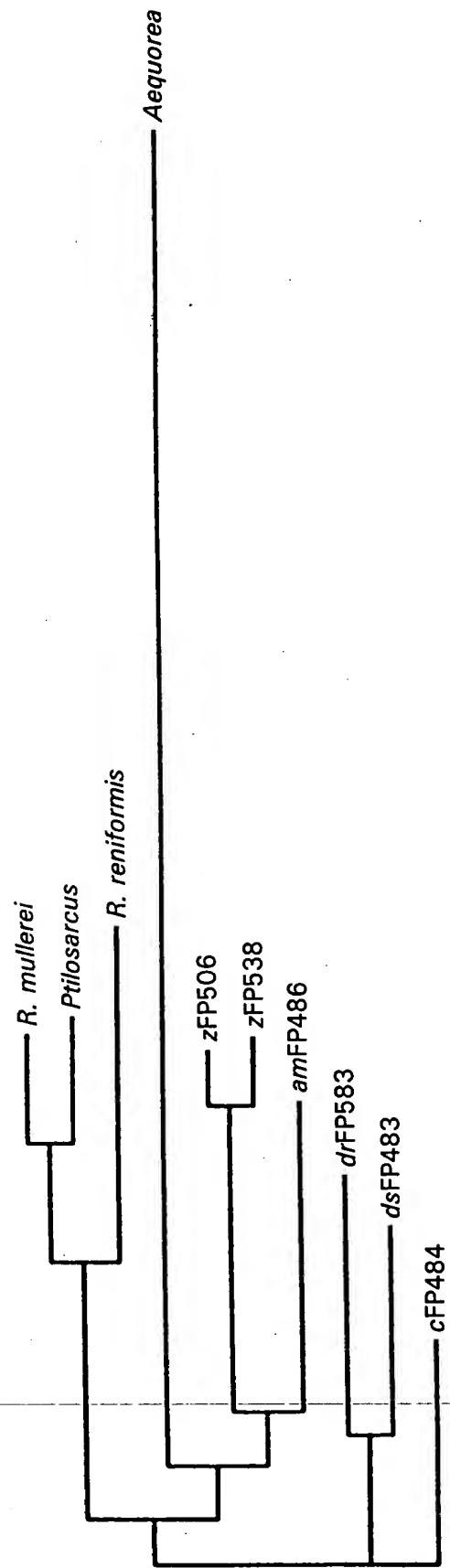


FIG. 1

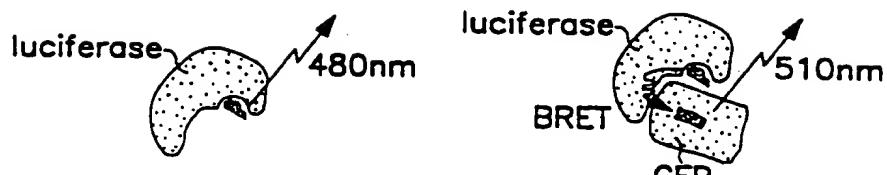


FIG. 2A

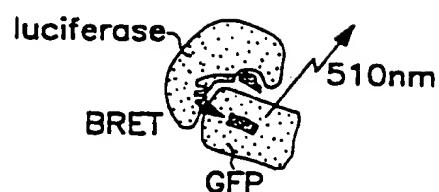


FIG. 2C

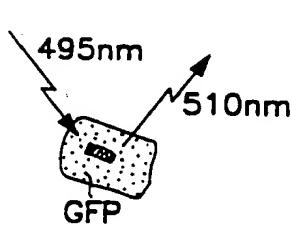


FIG. 2B

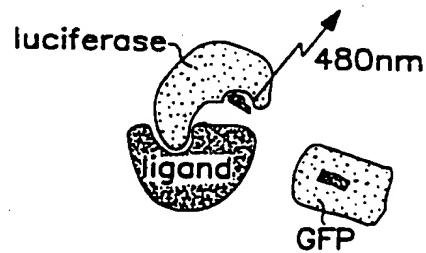


FIG. 2D

## BRET Sensor Architectures

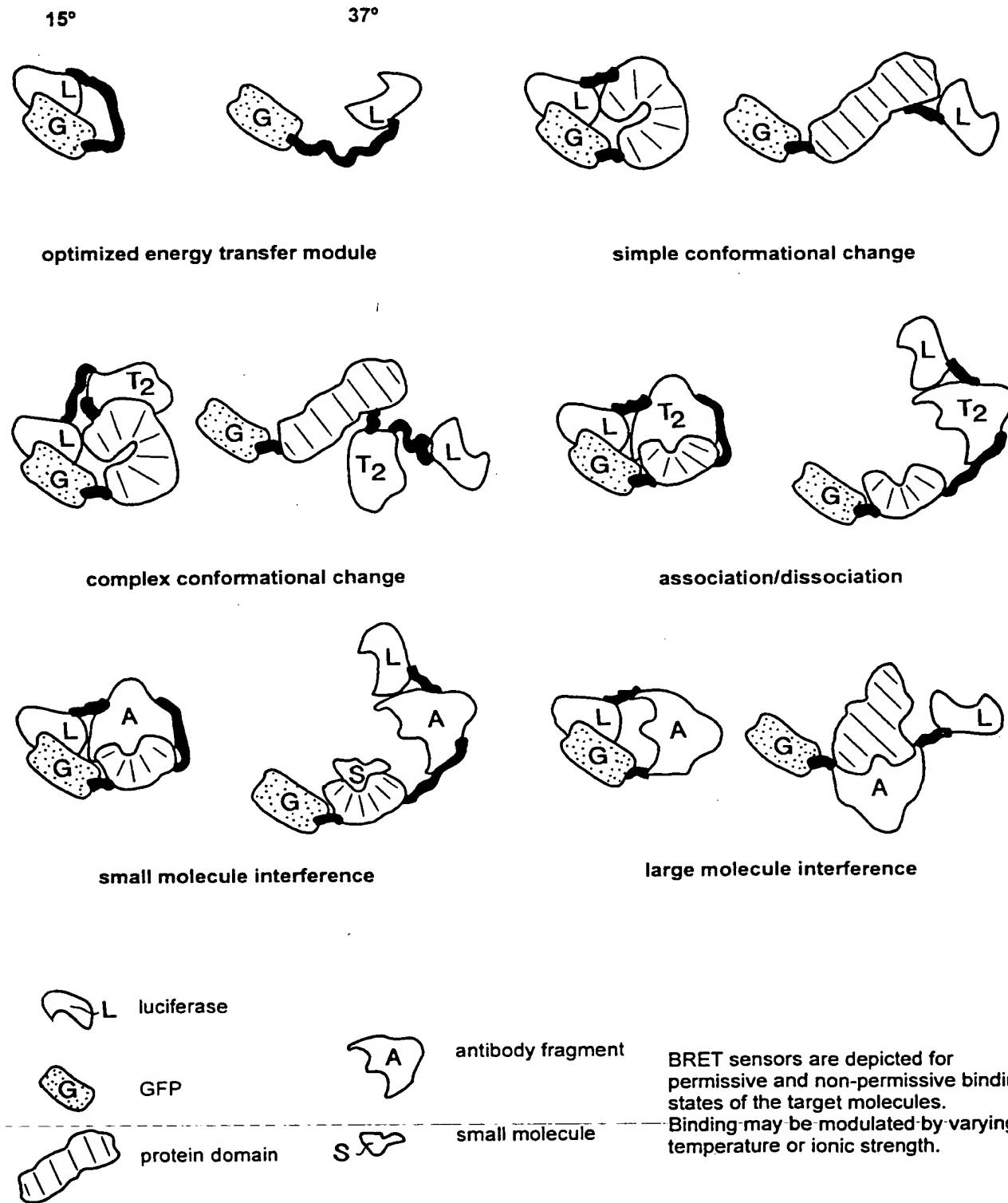


FIG. 3

Title: RENILLA REINFORMIS FLUORESCENT PROTEINS  
NUCLEIC ACIDS ENCODING THE FLUORESCENT PROTEINS AND THE USE THEREOF IN  
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Utilization of advantageous GFP surfaces with substituted fluorophores

	60	*	80	
RM-GFP	:	GAPLPFAFDIVSPAFAQYGNRTFTKYPNDIS--		: 83
Pt-GFP	:	GGPLPFAFDIVSIAFQYGNRTFTKYPDDIA--		: 83
RR-GFP	:	GAPLPFAFDIVSVAFSYGNRAYTGYPEEIS--		: 80
cFP484	:	GAPLPFSYDILSNAFQYGNRALTKYPDDIA--		: 83
drFP583	:	GGPLPFAWDILSPQFQYGSKVKHPADIP--		: 80
asFP595	:	GGPLPFAFHILSTSCMYGSKTFIKYVSGIP--		: 77
dsFP483	:	GGPLPFGWHILCPQFQYGNKAFVHHPDNIH--		: 80
amFP486	:	GGPLAFSFIDILSTVKYGNRCFTAYPTSMP--		: 82
zFP506	:	GGPLPFAEDILSAAFNYGNRVFTEYPQDIV--		: 80
zFP538	:	GGPLPFSEDILSAGFKYGRIFTEYPQDIV--		: 80

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FIG. 4

R reniform	---	MDLA KGLKEV PTKRIN LEGIV GDFHAF SMEGV GEGNILE GTQEV KISVTK GAPLPFA FDIVSV	*	20	*	40	*	60	*	63
R mullerei	: MSKQI.KNTC.Q.	[I]NNV.[I]NNV. [I]SY.V.[I]ASV. [I]SA.T.[I]NGE.FI. [I]RSS.NVI.F.RF.[I]VRM.E.	: P : P : P : P	C.K. F.N.L.Q.R. F.K.V.F.N.LMQ.R. RPY.HNT.LK.	: I : G : W.L.P	:	:	:	66	
Ptilosarcu	: MNRNV.KNT.	[I]NNV.[I]NGE.FI. [I]RSS.NVI.F.RF.[I]VRM.E.	X	F.K. V.F.HNT.LK.	G.	:	:	:	66	
drFP583	: ---	X	X	X	X	:	:	:	63	
R reniform	---	AFSYGNRAYTGY PEEEFSDYFLQS SFPEGGFTYERN IIRYQDGCTAIV KSDISIEDGKFTIV NVDFKAKDLVYR EYGSNF	*	80	*	100	*	120	*	129
R mullerei	: Q.TF.K.ND. Q.TF.K.DD.A. Q.SKV.VKH.AD.P. Q.Q	[I]A.M. [I]A.F. [I]A.F. [I]KW.	: P : P : P X	[I]TL.E. [I]L.FE. [I]VMNFE. X X	[I]LVEIR.N.IED. [I]AIMDIR.D. [I]VVT.TQS.Q. X X X	: N. : D. : C. X X X	: P : D. : Q. X X X	: KDLVYR.EY.GSNF	132	
Ptilosarcu	: ---	X	X	X	X	:	:	:	132	
drFP583	: ---	X	X	X	X	:	:	:	129	
R reniform	---	RRMGPVMQQDIV GMQPSYE SMYTNVTSVIG CIIIAFKIQTGK HFTYHMRTVYK SRKPVETMFLYHF	*	140	*	160	*	180	*	195
R mullerei	: PDD.KT.L. PSN.KA.L. PSD.KKTM.	[I]E.F.A. [I]V.F.V. [I]WEAT.RL.	: P : M. : E. : T.	[I]NGVLV.V.LVY. [I]SGVLV.VDLVY. [I]PRDGVLK.IHK.L.	[I]NS.YYSC.. [I]ES.NYYSC.. [I]KD.G.YLVEFSKI.I.MA. X	: K.LM. : K.F.R. : K.D.	: S. : E. : G. X X X	: GV.KEF.S.. : GG.KEF.E.. : APVQL.G.YY	198	
Ptilosarcu	: ---	X	X	X	X	:	:	:	198	
drFP583	: ---	0	0	0	0	:	:	:	195	
R reniform	---	IQRHIVKTNV DTASGYV QHETAIAAH STIKRIEGS TIP---	*	00	*	220	*	220	*	233
R mullerei	: ...E.Y. : .H.	EDGGF-E. SHNEDYTIEY.	: P : QLT.	...E. : E.	...QMTS.G. : QMTS.G.	: PL	: PL	: HEWV : HEWV	238	
Ptilosarcu	: ---	X	X	X	X	:	:	:	238	
drFP583	: VDSK.DI.	SHNEDYTIEY.	: P : E.Y.	TEGR.HLFL---	0	0	0	0	226	

5  
FIG

**Title:** RENILLA REINFORMIS FLUORESCENT PROTEINS, NUCLEIC ACIDS ENCODING THE FLUORESCENT PROTEINS AND THE USE THEREOF IN DIAGNOSTICS, HIGH THROUGHPUT SCREENING AND NOVELTY ITEMS.

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RHDFFKSAMPEGYYQERTTFFRDDGNYKTRAEVKFEG--	-DTLVNRIDPQGIDFEDGNILGHKLEINYNSHNIVYTMADKQKNGTK	162
-SDFYIQSFPAQFMERTLRYEDGGLVERSDINLIE--	-DKFVYRVENKESNFPDDGPVMQKTI-LGIPESEAMYM--	161
-ADYFVQSFPAQFFYERNLRFEDGAIIDVIRSDISLED--	-DKFHVKVIRENGFSNPGPVNQKAI-LGMEPSEENVYM--	161
-SDYFLQSFPEGGFLYERNLRYODGGIAWKSDISLED--	-GKFIVNVDNAKDLRRMGPVMQQL-VGMQPSSEMSYT--	158
-PDYKKLSFPEGFKWERMNFEDGGVMTVYQDSSLQD--	-GCFIYKVRFIGVNFSIDGPVMQKKT-MGWIASTERLYP--	158
-PDYKKLSFPEGFKWERMNFEDGGVMTVYQDSSLKD--	-GCFIYEWKFIVVNFSIDCPVMQRRT-RGWTEASSERLYR--	158
-HDYKLKSFPEGTYMERSMHFEDGGLOCITNDISLTG--	-NCFYYDIDKFIENFTINGCPVVQVKKT-TGWEPSTERLYP--	158
-ADYFKQSFPEGYSWERTMFDKGIVKWKSDISME--	-DSFIYEJRDGNNPPNGPVNQKKT-LKWEPSSTEIMYV--	161
-PDYFKQSFPEGFTMERTTYEDGGFLIAHQDTSLG--	-DCLVYKVNLLANNFPADGPVMQNKKA-GRWEPAETIWE--	155
-PDYFKQAFPDGMSYERTTYEDGGVATASWEISLKG--	-NCFEHKSTIFHGJVNEPADGPVMAKKT-TGDWPSFEEKMTV--	160
-VDYFKNSCPAGYTWRGSFLEEDGAVOLCNVDITSVKENCYIHKSKIFNGDNFEPADCPVMKKMT-TNVEASCKLMPVPKQGLIK--	-VDYFKNSCPAGYTWRGSFLEEDGAVOLCNVDITSVKENCYIHKSKIFNGDNFEPADCPVMKKMT-DNWEPSCKLMPVPKQGLIK--	162
asFP595		162
amFP486		162
zFP538		162
zFP506		162

Aequorea	-----
R. mullerei	-----
Ptilosarcus	-----
R. reniform	-----
drFP583	-----
drFP593	-----
dsFP483	-----
cFP484	-----
aFP595	-----
amFP486	-----
ZFP538	-----
ZFP506	-----
VNFTRHNIEDGSTVQADHYCQNTPIG-DGPVLLPDNHYISTQSALSKDOPNEKRDHMVLLFVTAGITTHGMDELYK---	238
GEVILWKLNSGNTISCHMKTLMKSKG--VVKEFPSYHEIQRHLEKTYVEDGGF--VEQHETAILAQMTSICKPLGLSIHLHWY	238
GEVDLWKLNSGNTISCHMKTLMKSKG--GVKEFPEYHEIHRHLEKTYVEEGSF--VEQHETAILAQMTTICKPLGSIP---	238
GECIAFKLQTGNTFTMRIVYKSKK--PVETMPLYHEIQRHLEKTYVEDGGF--PVTNNDKEFM-KVVEEHEILAKVWYRQKX	233
GETHKALKLDGGGM-LVEFKSITYMAKK--APVQLPQGYWYDSDSKLDITSHNEDYT-IVEQYERTEFJERHLFL-	226
GDTMHALRLEGGM-LVEFKSITYMVKK--PSVQLPQGYWYDSDSKLDMTSHNEDTY-VVEQYKQTCQJERHPPFTIKPLQ-	230
GDIHHTALTYEGGHIAQDIKIVYRRAKK--AALKMPQGYHYWDTKLVWTNNDKEFM-KVVEEHEILAKVWYRQKX	232
GDISHSLLLEGGHATRDFKSTIKAK--KVVKLKDYN-KVTLIYENAVARYSLIPSQA-----	231
GOSLMAKCPGGRHLICHLHTYRSKKPASALKMPGFHFEDHRIEIMEVEKCK-CYKQYBAAMCYRCDAAPSKLGHN-----	232
GDVIAFLMLQGGGNTRQFHISYKTK--KPVTMPPNHNVIEHRIARTDLDKGGN-SVQLTEHIAVAHHTSVVPF-----	229
GDVSPLLKLDDGGRARQFDTWIKAK--SVP SKMP EWHF IQHKLLREDRSDAKNQKWQLTBEHALAFPSALA-----	231
GDVSPLLKLDDGGRARQFDTWIKAK--SVPRKMP DMWEIQHKLLTREDRSDAKNQKWQLTBEHALAFPSALA-----	231

hydrophilic       hydrophobic  
dimerization surfaces

C,P  
not grouped

A, G  
small not

M, F, Y, W  
hydrophobic

L,L,V,M  
non-polar

N,Q,S,T  
or uncharged

K,R  
uncharged polar

D,E,H  
polar cl

60

104

100

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